ZOLLER

Caramel, an Intermediary Compound Formed in the Dry Distillation of Sugars

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CARAMEL, AN INTERMEDIARY COMPOUND FORMED IN THE DRY DISTILLATION OF SUGARS

BY

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A. B. Lenox College, 1911

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1913

1913
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THE GRADUATE SCHOOL

June 5, 1913

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Harper Filer Zoller

ENTITLED CARAMEL, AN INTERMEDIARY COMPOUND FORMED IN THE DRY DISTILLATION OF SUGARS

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry.

Geo. D. Beal — In Charge of Major Work

W. A. Noyes — Head of Department

Recommendation concurred in:

Committee on Final Examination

248:223

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CARAMEL, AN INTERMEDIARY COMPOUND FORMED IN THE DRY DISTILLATION OF SUGARS.

Introduction.

The investigation of carbohydrates, especially starches, dextrins and sugars, has been attractive to chemists ever since our knowledge of organic chemistry first became secure. This is no doubt due to the fact that carbohydrates are so widely distributed and so closely associated with all life processes- both plant and animal.

In reviewing the articles found in literature, regarding this deeply interesting subject, one becomes familiar with the researches of prominent chemists such as Gélis, Péligot, Völckel, Pellét and Reichenbach who worked between 1800 and 1850; since that time Fradiss, Trillat, Sabaneéff, Fischer, Abderhalden, Anderson,Nef and many others whose investigations are absolutely essential before arriving at a satisfactory conclusion regarding the properties and structure of these substances.

In the early researches one finds that more importance was attributed to the analytical side of the carbohydrate problem, i.e. the decomposition products and the compounds obtained by breaking down the linkage of the molecule. Now greater emphasis is placed upon the synthetic process of investigation, while a large amount of the data, secured by the former method,is too often ignored. In consideration of this last statement the writer is certain that the compound caramel has suffered such a fate. Not that the compound was never suspected, but the non-concordant data resulting from various investigations of the compound proves that many of the chemists were working at cross-purposes.

One investigator would secure a large amount of data by experimentation and then fail to qualify his results with facts obtained by other men who worked with similar substances, forgetting that these facts might essentially alter the basis for his conclusions. This point would become clearer should citation be made of the products resulting from the catalytic decomposition of sugars.

The purpose of this paper is to give a careful consideration of the compound and is based upon the data gathered by chemists who have worked with the substance together with the results obtained by the writer himself. An attempt will be made to correct the popularly conceived view of the term caramel. The word is of early origin and was first used by the Latin speaking tribes as carra-malla, meaning honey-cane or cane sugar. Then the French altered the word to caramel which indicated the product obtained by heating ordinary sugar in an open flask over a free flame. It was probably attributed to the brown, tarry residue resulting from this process. Or it may have been applied to the odor emitted by the decomposing sugar molecule. The process just mentioned was used by the French and other European peoples for disinfecting purposes. Just when the word was first adopted by the English is not known, but up to the present time the usage of the pure French term is retained. The Germans probably derived their " karamel " directly from the French. During the last century caramel has had such an extensive commercial use that the conception has been gradually changed to include that part of burned sugar which could be used as a coloring agent. Its use as an adulterant in elixers, syrups, liquors, wines, vinegars, extracts coffee-berries and sometimes in milk is only too well

known.to the modern food analy st. The physiological chemist still uses the term to indicate the burned sugar odor and brown colorations produced when starches, sugars, etc are heated. This is probably due to the fact that caramel in its crude form was used as a therapeutic, because of a large content of formaldehyde in polymerized form .

An extended review of the literature regarding the subject is made. Perhaps no subject in Food Chemistry has received more thought, or has been tampered with by investigators of all the leading countries for such a long period of time and has yielded such apparently fruitless returns. The works of Péligot, Völckel, Gélis, Trillat, Stolle and Zsigmondy will be quoted as representing the most efficient researches to date. Many other articles have appeared but they have to do primarily with the detection of caramel in fraudulant adulteration of food-stuffs. A study of the decomposition products, obtained by dry distillation, to show the analogous properties of carbohydrates will broaden our knowledge as to the exact time at which we may expect the compound caramel to appear.

In the second place methods for the isolation of the pure compound from cane sugar(Sucrose) and Glucose will be given in full. The properties, chemical and physical, of these two substances will be mentioned. The cleavage products of the pure compounds of caramel, so far as they have been studied, will be considered.

The third part has to do with the comparison of Sucrose and Glucose caramel in elementary composition and properties. From these considerations it will be evident that under similar conditions Sucrose and Glucose yield different compounds of caramel.

A careful analysis of prepared derivatives has been made and the molecular-weights of the respective caramels roughly determined. The extreme difficulty of working with amorphous compounds is well known, so it will be only after several years work that a complete knowledge of the structure of the compound caramel will be entertained

We have experimented upon a number of the simpler sugars of the carbohydrate family, especially the mono-, di-, and tri-saccharides. In all cases varying amounts of caramel was noted. A statement as to the possibilities in regard of this fact will be found in the preceeding pages.

A short discussion of the methods for detecting caramel in food-stuffs is included in the closing paragraphs. The tests which have been in vogue for the last twenty years have proven both unsatisfactory and unreliable. It was while engaged in examining certain spirits and vinegars for the presence of caramel as an adulterant that we became convinced of the importance attached to this substance and the increasing necessity for a reliable and conclusive test for its presence.

1.

Is " Caramel " a Compound?

We will endeavor to formulate a satisfactory definition of caramel from the investigations previously conducted and from our experiments which corroborate most of the early researches and in some measure extends our knowledge of the substance. The generally accepted view of the term caramel is in effect misleading, and quite opposes the findings of the investigators. To the layman it is a relative term for a pleasant smelling and tasting substance; to the average chemist it indicates a varying

mixture of compounds. Whether or not the researches have been considerated unreliable, owing to the lapse of time intervening between them, the investigators were sincere in their undertakings; though their methods may have been crude and undeveloped.

We believe that caramel is a compound, in fact a very stable compound, which may be obtained from the sugars of commerce, as well as from other sources, by simply heating them to the temperature necessary to produce the greatest quantity of soluble coloring-matter. All sugars and substances have "caramelization points" characteristic for that specific sugar or substance. We find it more convenient to change the prevalent and unfounded conception of caramel than to suggest a new name. The different caramels from the various sugars may be indicated by prefixing the name of the carbohydrate from which it was obtained as, Sucrose Caramel, Raffinose Caramel, etc.

The earliest record which we can find of investigations upon the products of distilled sugars is mentioned in the original article of Péligot(1). He states that Cruickshank, an English Chemist, attempted to determine the weight of the various substances resulting from the distillation of cane-sugar. Caramel had been used in commerce previous to Peligot's time, both as a coloring agent and as a therapeutic. He realized that there must be some definite compound formed, when carbohydrates are decomposed, which is soluble in water yielding a sepia colored solution. He found that when Sucrose and Glucose are exposed to the action of heat the following changes appear. When the temperature of the bath has reached 180 degrees the sugar(Sucrose)

(1) Ann. de Chemie et Physique. 2nd ser.67- 172- 1838.

begins to melt and as the temperature rises it becomes yellow; and when 210 degrees is reached the color gradually deepens to a dark-brown. At 180 degrees it lo ses no water but a little past this point dehydration begins. Evidently what Peligot witnessed at this point was the formation of Barley-sugar, a compound resulting from a partial dehydration of Sucrose between the temperatures 180° - 190°C. " On raising the temperature to 210°- 220° and maintaining it near this point for some time, the sugar swells and a spontaneous reaction takes place. It acquires a brown tint which becomes deeper and deeper. It does not give off the least quantity of a permanent gaseous product but yields considerable quantities of water-vapor, traces of acetic-aldehyde and an oily substance which has the odor of burned sugar". Our recent advance in knowledge and methods of manipulation have revealed that very permanent gases are evolved under similar conditions, and the disinfecting qualities of burned sugar have their foundation in this fact.

After the frothing of the sugar had ceased Péligot removed the distilling-flask from the bath, dissolved the contents which were soluble in water, and precipitated the coloring-matter with alcohol. The precipitate was dissolved again and reprecipitated with alcohol inorder to secure as pure a product as possible This product was now dried and desiccated. It was found to be a slightly-bitter, shiny, amorphous substance, easily soluble in water and could not be fermented. When treated with ammoniacal lead-acetate it yielded a copious precipitate and with barium-hydroxide a similar reaction. An elementary analysis of the desiccated substance gave Peligot the following percentages-

Carbon---46.6 - 46.6- 47.5 -46.9%

Hydrogen- 6.1 - 6.1- 6.2 - 6.3%

which gave him an empirical formula of $C_{48} H_{36} O_{18}$. The results from his analyses proved to him his belief that it was a definite compound of unvarying composition. Since he had no method at his disposal for accurately determining the molecular weight little credence can be placed in the approximated empirical formula. He found hydrogen and oxygen in the porportion to form water, thus indicating a compound of carbohydrate nature. He ended his report with the statement that upon continued heating the caramel gradually decomposed yielding more water and an insoluble compound. This insoluble compound, when analyzed, was found to be a carbohydrate. It possessed no value as a coloring agent. Extended heating left only pure carbon as residue.

The next research of importance and one to throw more light upon this compound is that of Völckel(1). His chief aim was to study the compounds formed in the dry distillation or organic substances. The ordinary sugars were examined at length,and in his article, "Fortgesetzte Untersuchungen uber die Producte der trockenen Distillation organisher Körper", we find the first proofs of the evolution of formaldehyde simultaneously with caramelization. The gases were collected in water: the solution neutralized with sodium-carbonate and then distilled. After extraction of the liquid with ether, dry ammonia gas was added and white crystals of hexamethylene-tetramine resulted. Furfural and pyroligneous-acid were isolated from the condensed gases. In the distilling-flask, caramel and a substance called "assamar" were found; formaldehyde and free carbon were also present.

(1) Annalen der Chemie. vol 85-86 -59 -1853.

Assamar is a term used to designate that part of the residue, after the caramel has been dissolved out, which, when treated with sodium-carbonate on a hot-water bath, yields a thick syrupy liquid. Reichenbach, working contemporaneously with Völckel, first applied the term and he seems to have confounded it with caramel. However the word seldom appears in the chemical literature of today. Völckel believed the empirical formula to be $C_{12} H_9 O_9$; but neglects to state whether or not he examined the purified compound.

In 1858, five years later, there was published an article by Gélis(1). This man made three distinctions in the compounds of caramel, extending the word to include those compounds which resulted from increased molecular weight but differed as to solubility. The "caramelene" and the "caramelin" were insoluble in water to the extent of the "caramelane". The investigation of the properties of purified caramel was carried to a further point than had previously been attempted. He found that it was not precipitated by neutral metallic salts; it reduced the metals copper, gold and silver in alkaline solution and was amorphous. Nitric acid converted it into oxalic acid. His elementary analyses revealed a compound similar in composition to that of Peligot, although he is not certain as to its correctness for he writes "Cette formule est exactement celle qui a ete attribute par M. Peligot a son caramel normal; mais il est evident que le s substance que j'ai analysee n'a aucun rappart avec la sienne". His results corroborate wall those of Peligot-

Carbon------46.64	-46.17%
Hydrogen---- 6.62	- 6.44%

(1) Ann de Chemie et de Physique. vol 52 -352 -1858.

Peligot, we remember, described his compound empirically as $C_{48} H_{36} O_{18}$ while Gelis gives $C_{12} H_{8} O_{8}$. We are inclined to believe that Peligot's caramel was more carefully purified than was Gelis'. In either case little attention was paid to the contaminating sugar and other products which are precipitated along with the caramel by alcohol. The true worth of Gelis' investigations lies in the distinction which he made between the caramel formed from Sucrose and that formed from Glucose. He was the first chemist to note the dissimilarity in the physical constitution and properties of the two compounds, but his records do not show that he made any analysis to show the difference in chemical structure. The experiments described above were made upon Sucrose.

It is not till 1885 that Amthor's discussion of the data secured by previous investigators appears. In this article Amthor proposes the paraldehyde test which has been more used than any other for the commercial detection of caramel. He has given no basis for the efficiency of this test. More consideration will be given to this test in the latter part of this paper.

A series of interesting investigations upon the cleavage products of Sucrose caramel have been reported by Stolle(1). He used yeast to ferment the sugar which remains in the unconverted form after the caramelization has been stopped. The caramel was dissolved from the residue by water, yeast was added and after all traces of the sugar had been removed the solution was filtered and evaporated on a water bath. Stolle found that it dissolved easily in water, was amorphous and had a melting-point range between 134-136 degrees. The elementary analysis gave 46.2% Carbon 6.4% Hydrogen and 47.4% Oxygen. This he says is identical with

(1) Zeit. fur Unter der Nahr. und Genuss. page 357 -1900.
Rubenzucker Ind. pages 800 and839 -1899.

Gelis' "caramelan", $C_{12} H_{18} O_9$. Since no definite molecular weight determination was made we hardly see how he is certain of this empirical formula. He now employs some of the more modern reactions to show the characteristic properties of the compound. When heated with sodium-acetate and acetic-anhydride a " tetra-acetyl Karamelan" was secured- $C_{12} H_{14} (C_2 H_3 O)_4 O_9$. Benzoyl-chloride and potassium-hydroxide yielded a brownish nodular precipitate, which he thought was a mono-benzoyl derivative. It is by means of this derivative that we sought to differentiate between sucrose and glucose caramel as well as study the probable grouping. Stolle mentions the work of Vogel who found that caramel in water solution influenced absorption in the blue side of the spectrum(1). In this connection Vogel prepared absorption spectra for 0.1 - 0.2 -0.5 and 1.0% solutions of caramel.

In studying the cleavage products of his purified caramel Stolle first employed 3% HCl. An 18 hour digestion of caramel with this concentration of acid, upon a hot-water bath gives an almost colorless solution together with a brownish precipitate. This insoluble body amounted to 30% of the original caramel and was not studied further. In the liquid portion there remained a substance resembling a reducing sugar. It could not be induced to crystallize. It tended toward bi-rotation. The osazone was prepared and melted at 197C. It corresponds to an osazone of a hexose. In acetic-acid the osazone was found to be laevo-rotatory. With 3% sulphuric-acid he perceived similar phenomena, and secured similar products. Stolle purified the sugar from the

(1) Vogel; Spektral analyse iridischer Stoffe, 2 aufl.S.52.

cleaved caramel by use of methyl-alcohol and obtained it in crystalline form. Both the anhydride and the hydrate were isolated, the values of the rotation constants determined, the osazones prepared and the melting-points of the osazones determined. From these studies he concludes that the soluble compound resulting from the action of dilute mineral-acids upon the pure caramel must essentially be an aldo-hexose. Later Stolle found that the action of nitric-acid on the insoluble, brown precipitate remaining in the digestion-flask, after action of hydrochloric or sulphuric-acid, converted it into oxalic-acid. From this he decided that it must be a humus-body. This examination of the cleavage products is valuable but Stolle's conception of the formation of caramel as reported in his researches is radically wrong. In writing the reaction

$$C_{12}H_{22}O_{11} + \text{heat} = C_{12}H_{18}O_9 + 2H_2O$$

he ignores the gaseous compounds which are evolved simultaneously. Remembering this fact he would cease to look upon caramel as merely dehydrated sugar. During the action of heat for a considerable length of time a rearrangement probably takes place within the sugar molecule whereby condensations have given rise to numerous compounds -some of much greater molecular-weight than sugar and some considerably less. The question to be settled is " whether there may or may not be simultaneous polymerizations and condensations taking place."

The next work of interest is that of Trillat(1) on "The Presence of Formaldehyde in the Products of Caramelization". He spent much time in searching for the basis of formaldehyde in vegetable distillation and combustion. His careful analyses

(1)Bul. de la Societe Chimique de Paris. 90 -681 -1906.

of the air of smoke-laden sections of France are well known. Perhaps no one person has broadened our knowledge of formaldehyde to a greater extent. But that which interests us most in this connection is his reports on the amount of formaldehyde formed in the dry-distillation of sugars. A great variety of carbohydrates were used and the amount of formaldehyde formed per kilogram of substance was determined. Mention should be made of the catalytic effect that certain metallic containers have upon the amount of formaldehyde produced. Glass was found to yield a normal amount but when a copper retort was employed it increased the output 200 times. In distilling cane-sugar he found 7% formaldehyde. This sugar yielded a larger percentage than any which he examined. A portion was evolved as vapor and part remained in the flask in the polymerized state, as para-formaldehyde and tri-oxy methylene. We might state that Trillat's knowledge of the early and later researches on caramel must have been limited for he still considered it to be the entire residue remaining after the distillation of the sugar. Translating from the original article we note the following-

" On heating sugar to 125° C immediately traces of formaldehyde appear. At 150° C the evolution is stronger and by briskly raising the temperature it is instantaneous. On repeated analysis of the gas I have obtained-

Formic aldehyde -------	.2 to 5.7%
Acetic "	----------
Benzoic "	.5 " 1.4
Acetone	.1 " 5.0
Methyl alcohol	----------
Acetic acid	1.0 " 3.0
Phenolic derivatives	1.0 " 3.0 "

This is the first systematic analysis of its kind. Though he

does not state definitely as to the sugar employed in the above examination one infers that it was sucrose. Peligot seems to have been the only investigator, other than Trillat, to attach the least importance to the gases evolved.

In determining the percent of formaldehyde remaining in the distilling-flask after caramelization Trillat used various methods. In all cases similar amounts were revealed. He even went so far as to study the antiseptic properties of "caramel" by proving its efficiency as a milk preservative and Bacillus Coli exterminator. These latter facts have little significance except as they prove more conclusively that formaldehyde remains in the residue after caramelization. Pure caramel, we have found is not an antiseptic, neither does it contain the least traces of formaldehyde.

There are numerous other short articles to be found in the literature of both this and foreign countries concerning caramel and its use as a coloring agent, but they throw no light whatever upon its characteristic properties. Before turning our attention to the experimental part it might be well to mention some ideas expressed by Sabeneeff(1) in his article "Cryoscopy of Caramel". The real value of this paper is concerned with the methods used for purification. He was the first investigator to use dialysis as a means of separating caramel from its impurities. No statement as to the membrane employed is made. Sabaneeff thought the molecular-weight of caramel was about 3000. and that it was not a carbohydrate because carbonic-anhydride was given off. Evidently he wished to consider caramel as merely dehydrated sugar, but, finding that the sugar molecule was ruptured, he concluded that it must be classed in another series.

Had he analysed the gases he would have found that the carbonic-anhydride was evolved in the form of definite organic compounds comprising those mentioned by Peligot and Trillat. This chemist calls attention to the fact that dialyzed caramel is capable of undergoing a species of fermentation by virtue of which he finds an increase in the molecular weight. He also states that a solution ofthe caramel is affected by the action of heat and light and that these effects tend towards an increased depression in the freezing-point of the substance. Sabaneeff calls the process taking place in fermentation a dehydration and in the latter case a hydration.

The results from these investigations lead us to the following inferences,

(1). Sucrose caramel is a compound of definite composition- Carbon 46.5%; Hydrogen 6.0%; Oxygen 47.5%. It is easily soluble in water and is precipitated from water solution by strong alcohol. It yields a copious precipitate with ammoniacal lead-acetate and Barium-hydroxide. Reduces metals in alkaline solution. May be acetylated or benzoylated yielding difficultly soluble compounds. A precipitate results from treatment with phenylhydrazine in acetic-acid solution. (Peligot, Volckel and Stolle).

(2). There is a probable distinction between the sucrose and glucose compounds.(Gelis). Carbohydrates, other than simple sugars yield a similar substance.

(3). In the process of distilling sugars to obtain caramel other products are simultaneously evolved. viz, benzaldehyde, acetic-acid, acetone, formaldehyde, phenolic-compounds etc.(Trillat)

(2)

EXPERIMENTAL PART

Our attention was first directed towards the preparation of caramel in its purest form. In the first place, because of its cheapness, sucrose was employed to study the best methods of production. A bath of cotton-seed oil was used for nearly all the preparations, although a bath of Wood's Metal was once used and it proved to be very satisfactory. The sugar is placed in a large, round-bottom Florence-flask of Jena glass. A one-liter flask is best used for 100gm of sugar. This is placed in an oil bath which is at a temperature of 210degrees. This temperature is maintained for a period of 30 min. with cane-sugar. At the end of this period most of the sugar will have been decomposed and no free carbon formed. The residue is then extracted with water which dissolves out the caramel, unconverted sugar and some soluble compounds formed. The solution is thoroughly filtered to remove any free carbon and other insoluble compounds which are always present. When 500gm of water is used to dissolve the residue from 100gm of sugar the resulting solution is a deep brown. It is exceedingly bitter, slightly acid in reaction and the presence of a small quantity of sugar is unmistakable. These contaminating substances must be removed before a satisfactory examination of the desired product can be made. The methods employed by the previous investigators, who purified the product, were- precipitation by alcohol, fermentation and dialysis. Now alcohol will precipitate the compound caramel when large volumes are used but it also precipitates any sugar present and this precipitate is liable to occlude other substance s present.

In the trial precipitation with alcohol we employed one liter of 95% alcohol to 100c.c. of caramel solution. This precipitate was collected upon a filter, washed with alcohol and redissolved in water. It was then evaporated upon a hot water-bath and yielded a residue which seemed to be free from taste except a slight bitterness. In water it was completely soluble and gave a solution much darker and slightly red.

In the next purification we used common compressed yeast. Two-hundred cubic centimeters of caramel was placed in a large flask fitted with a proper outlet tube. The yeast, one cake, was now added and the whole apparatus kept at 35 degreesC for three days. Upon the expiration of this period the fermentation was found to be complete and that the color of the solution had not changed in the least. To free the mixture from yeast-cells a Buchner funnel with suction was employed and a layer one-fourth inches thick of Fuller's Earth(infusorial earth) was used over a filter paper in the funnel. This gave a splendid filter of very fine pores and was found to be preferable to the ordinary porcelain apparatus. After filtration the resulting solution was evaporated to dryness. A notable difference was observed in the physical properties of the evaporated mass in comparison with those presented by the alcohol precipitation. It was found to be extremely bitter and acid to the taste. It is quite as readily dissolved by water but the solution is slightly acid and still possesses a bitter taste. This can be explained by the fact that in fermentation only the sugar is removed while any other polymerized or condensed products formed are still present.

(1).From page 13-
Journal of Soc. Chem. Ind. vol 13 -1076 _1894.

While searching for a more suitable method to remove the sugar and other materials Sabaneeff's scheme suggested itself. For ease of preparation we decided to use collodion as a membrane instead of parchament. It has been held for some time that collodion affords a semi-permeable membrane but was difficult to form in a utilizable shape for osmotic work. In order to form a tenacious membrane a collodion bag was made by pouring the pure collodion in a large-mouthed Erlenmeyer flask and allowing a thin film to line the sides of the flask. This film could then be peeled off with the aid of water and extreme patience. This method produced a bag of considerable tensile strength and "water-tight".

Later we found it more convenient to prepare our membranes in the following manner. A tall beaker of 1000 cc capacity is employed. Into this about 20 cc of collodion is poured. The beaker is then rotated so as to obtain the collodion in a uniform lining on the inner walls. Now invert the beaker and continue to rotate between the palms of the hands. This caution hastens the evaporation of the ether. When the odor of ether is no longer noted (usually 15 min.) carefully run the point of a knife around the mouth of the beaker and loosen the collodion. By peeling the membrane from the side of the beaker a bag can be obtained which will support the weight of an equal volume of water. The bags should be kept in water when not in use otherwise they quickly dry and crack.

About 200 cc of caramel solution is now poured in one of these bags, a small capillary tube fastened in the shirred top and the bag and tube supported by a clamp in a vessel containing

distilled water, with the water and the solution in the bag at the same level. Almost immediately streams of sugar solution begin to pass into the water, while water passes swiftly into the bag causing an increasing volume. This is allowed to continue for twelve hours when the water is replaced by a fresh portion. After dialysis has continued for 48 hours the caramel is tested for sugar and not even a trace has been found in any of our examinations after this period of time has elapsed. The characteristic tests with resorcin and gallic-acid for formaldehyde fail to reveal any in the caramel solution in the bag after the above method of purifying has been used. Neither can acetic-acid be detected. Qualitative tests upon the water solution outside of the bag reveals, in the early stages of dialysis, a strong positive test for formaldehyde, reducing-sugars and acetic-acid. After 30 hours have elapsed the tests for formaldehyde are very faint; and in most cases, with Fehling's solution and other tests for sugars, they are negative at this point. Of course the amount of sugar present at any time depends upon the degree of caramelization but in this respect 36 hours are usually sufficient to remove the last traces of sugar. In the last few hours of dialysis a portion of the caramel travels through the membrane into the water on the outside. We find after evaporating the dialyzed caramel that a residue remains which possesses very little bitterness and is quite like the caramel secured by precipitation from alcohol. However in this case it seems to be entirely free from contaminating substances.

The mechanism of the above dialysis depends upon the selective properties of the various substances in solution and the relative

speed at which they permeate the membrane. The collodion bags can be used for dialysis but once , since they become somewhat weakened in the lower portion at the end of the experiment. In a recent book on Colloid Chemistry we find a short treatise on caramel considering it as a colloid. Zsigmondy(1) investigated this property and we find it of importance in this connection. In one place we note the following statement-

"Karamel ist etwa 600 mal weniger dialysabel als chlornatrium und 200 mal weniger als zucker".

He gives a formula of $C_{24}H_{15}O_{15}$ for dialyzed caramel obtained from sucrose.

It is evident from the above results that the compound is best purified by dialysis. In studying the preparation of glucose caramel it was found that the above method is applicable except that a longer time is required for complete caramelization.

We will now trace the evolution of caramel from the pure sugars and study the accompanying phenomena. Equal volumes of both sugars are placed in proper distilling-flasks and these connected by tubes to Woulff bottles containing distilled water. These are to catch the evolved gases which are mostly soluble in water. The distilling-flasks are now placed in an oil-bath and heat applied. No action takes place in either flask until a temperature of 140 degrees is reached when the glucose begins to melt; at 180° it loses considerable quantities of water and gases are evolved. This evolution of vapor continues for about 15 min. while the temperature is between 185° and 195°. Now as the temperature in the outer bath is raised to 210°-215° and maintained at this point for 45 min. longer, there is a gradual diminution in the quantity

of gases given off. The longer the flasks are allowed to remain in the hot oil-baths the stronger becomes the odor of benzene compounds in the gases. A thermometer placed on the inside of the flasks,in contact with the boiling mass, fluctuates between 150°-180° and finally reaches 190° where it remains constant till the end of caramelization. When the temperature of the outer bath reaches about 200 degrees the glucose begins to froth and this continues until the flask is nearly filled. A gradual change in color takes place. From a grayish-white froth it passes through a yellow into an orange, thence to golden-brown and finally a brownish-black. This last coloration is obtained during the last 15 min of the process. At this point the frothing gradually ceases and the product is removed from the bath and allowed to cool.

In case of sucrose the behavior is different. It does not melt until a temperature of 160 degrees is reached; then it melts slowly and gases are not evolved to any noticeable degree until 190°. Less water-vapor is given off and very little frothing is observed. It boils steadily with evolution of vapors rich in benzaldehyde and acetic-acid odors. At 180 degrees sucrose forms an allotropic variety known as barley-sugar. This is yellow in color and is of little importance in this connection, except to show that the sugar molecule alters early during the proceedure. In the caramelization of both sugars part of the water is condensed at the constriction in the neck of the flask and returns to the boiling mass. The absorbed gases color the water in the Woulff- bottles a dark yellow in case of sucrose and a lighter color in case of glucose. Both solutions smell

(1)Kolloid Chemie und Photo. by Luppo-Cramer. page 8 -1908.

strongly of "burned-sugar" - mainly benzaldehyde, formaldehyde, acetic acid etc. The same odor is apparent from the residue in the flask; after dialysis a similar odor is noted in dialyzate bathing the outside of the membrane. Purely qualitative analyses performed upon the solutions of dissolved gases reveal similar products as found by Trillat. The sucrose products were richer in benzaldehyde and formaldehyde than were those from glucose. This is probably due to the size and character of the sucrose molecule.

Both residues upon cooling are black, shiny and hard. Upon addition of like quantities of water we found the sucrose to possess a coloring power almost ten times as strong as glucose. After purification this same fact held true. This finding would seem to dispute the announcement of Beringer(1). He writes in the above journal that glucose is supposed to form the greater part of caramel now upon the market. If such is the case then it is through the ignorance of the manufacturer as to the qualities of the caramel from the respective sources.

The Characteristic Reactions of Glucose and Sucrose Caramel Purified by Dialysis.-

Analogous results were obtained with the alcohol purified variety, and parallel experiments were conducted throughout the entire investigation to ascertain if this held true in all cases. In the first place absolute alcohol precipitated the sucrose compound more completely than it did the glucose. It required such large amounts of alcohol that this seemed cumbersome as well as expensive. Amyl alcohol partially precipitated an alcoholic solution of caramel. Methyl alcohol produces no precipitate.

(1)Jour. A. Ph. A. — 1912, page 219.

Both glucose and sucrose caramels as prepared are amorphous compounds, dark-brown when powdered and possessing a neutral reaction when in solution. They are practically free from bitterness, odorless and possess no sweet taste. The melting-points cannot be obtained since we have to do with amorphous compounds. However the two varieties yield quite distinctive liquefaction-points. The compounds are so colored that a definite demarcation is impossible. The following points were obtained with a great deal of care-

Sucrose caramel,,.....158 -165°C.
Glucose " .....132 -135°C.

We perceive a marked difference between the two varieties as revealed thus far in the preparation and liquifaction-points, but in the chemical derivatives formed we find a still greater difference. The two compounds thus far studied give such similar test reactions that a method evolved for the detection of the one will answer as well for the other.

Upon treating the purified compounds with Fehling's Solution we get a positive copper reduction after boiling for some minutes in a water-bath. The glucose is slightly more rapid in the reduction than is the sucrose caramel. This leads us to look upon them as carbohydrates and possessing the carbonyl grouping. With ammoniacal silver-nitrate an immediate reduction is noted (Tollen's aldehyde test). Another test, quite distinctive for aldehydes in the absence of the lower alcohols and acetone is the iodoform reaction of Liebig. In the case of caramel we get a positive test, more pronounced for the glucose compound than with the sucrose. The test is performed by adding a small portion

of a strong solution of iodine in potassium-iodide, a few drops at a time, to a solution of caramel made alkaline with sodium hydroxide. When the iodine ceases to be decolorized add a slight excess and then add more alkali until the solution looses the dark iodine color. After allowing to stand for some time a distinctive odor of iodoform results and crystals are deposited on the bottom of the container. The test is best performed in the cold. Dilute solutions of caramel are employed otherwise they would be too dark to observe the desired changes.

We also studied the reaction taking place when caramel is treated with phenylhydrazine. Amthor was the first investigator to try the action of this organic compound upon caramel and he ceased with the securing of a precipitate in case of sucrose. In all hydrazones prepared from ordinary sugars the osazones or hydrazones have been secured in crystalline form. Here, however, we meet a different form. Since we are dealing with amorphous compounds we secure an osazone with amorphous qualities and cannot be induced to crystallize. In brief the test was applied as follows. Concentrated solutions of caramel were employed in b both tests. Phenylhydrazine-hydrochloride and sodium-acetate were first used but this was superceeded by pure phenylhydrazine in 50% acetic-acid. One part of phenylhydrazine to four parts of the 50% acetic-acid seemed to give the best results. 10 cc of a solution in the above porportions was added to 25 cc of the sucrose caramel(in the cold). Almost immediately a dark-brown turbidity resulted and upon heatimg in a water -bath for one hour the osazone settled upon the walls of the containing vessel.

With glucose caramel a similar precipitate resulted. We examined the precipitates under the microscope for crystalline structure but found none. Attempts at crystallization in the following manner were fruitless. The liquid remaining in the flask may be decanted from the precipitate with ease. Of the solvents only glacial-acetic-acid completely dissolves it; nitro-benzene and benzene possessing some solvent action. To remove the acetic-acid from the osazone we boiled the latter with alcohol which eliminated all traces. The remaining phenylhydrazine was removed by extracting with ether. By repeated washing and subsequent drying it was obtained quite pure. To further purify we dissolved the osazone in acetic-acid and reprecipitated with water.

The derivative was amorphous; a qualitative examination for nitrogen by means of the prussian-blue test gave positive results. Glucose caramel yielded a magenta-brown compound while the sucrose caramel derivative was almost black. A quantitative examination for nitrogen gave results as reported in the third part of this paper. We are sorry that time allowed only for the determination of nitrogen in the glucose derivative. Further attention will be given to the osazone in another paper. The formation of the water-insoluble osazone is further evidence that the two caramels studied are carbohydrates and possibly of the hexose species, since hexa-carbohydrates yield insoluble osazones.

By treating solutions of these caramels with basic-lead acetate the entire compound was precipitated in the form of a flocculent mass - light-brown and amorphous. No distinction between the two varieties except a somewhat darker salt is obtained in case of the glucose caramel. Barium-hydroxide gave a similar precipitate. The analysis of the barium and lead salts

are given much attention in the report of Gelis(1).

The next examination we made was for saturation. Dilute solutions of potassium-permanganate when added to unsaturated compounds is immediately decolorized. Bromine-water is similar in reaction. Now when potassium-permanganate is added to solutions of caramel some time elapses before it is decolorized or any activity is noted. After allowing it to set for a few hours we found that a flocculent, black precipitate had settled in the lower portions of the test-tube. In other trials we added more caramel solution and used greater quantities of permanganate. Upon standing for a period of 12 hours we noted that both the glucose and sucrose varieties could be precipitated from solution, and only a light yellow coloration will be left in the remaining liquid. The precipitate were filtered, washed, dried and then ignited on a platinum foil. The carbon burned slowly from the mass leaving about one-third of the original amount of the substance. The residue was found to be an oxide of manganese, probably Mn_3O_4. The deduction made from this reaction is that after standing in contact with the caramels for some time the permanganate first saturates the double-bonds and finally oxidizes the entire compounds. It is difficult to ascertain for certain whether decolorization is immediate or not owing to the intense color of the caramel. This reaction has not been sufficiently studied to determine in what form the oxidation products exist.

With bromine-water a gradual oxidation proceeds. Whether we obtain a compound in which the bromine has acted by addition or direct substitution is not known. But the analysis of the precipitate, which results from long standing, indicates that a

(1). Ann de Chemie et de Physique. vol 52 -352 -1858

very small amount of bromine is present.

When a solution of sodium acid-sulphite is added to glucose caramel we find the color of the caramel almost disappears and a strong effervescence of sulphur-dioxide is noted. A general cloudiness fills the liquid suggesting the suspension of small particles, probably sulphur. When sucrose caramel is treated in a similar manner there is not such a pronounced decolorization though the depth of the color is noticably diminished. This sulphite test is given by Noyes and Mulliken as a characteristic reaction and entirely distinctive for the $CH_3>CO$ grouping. The principle of this reaction is the simple addition of the SO_3 radicle to the oxygen of the carbonyl.

The following reaction is based upon the affinity existing between acylated compounds and the hydroxyl groups of organic substances. Benzoyl and acetyl were the acyl compounds employed. Concentrated solutions of the pure caramels were made strongly alkaline with 50% sodium-hydroxide. This solution was placed in a large sepratory funnel and benzoyl-chloride added in sufficient quantity. The mixture was kept cool in a mixture of ice and water and was shaken at intervals to insure a complete reaction. Both caramels are entirely precipitated and form a brownish nodular precipitate. The only satisfactory solvents are benzol, nitro-benzol and glacial acetic-acid. In purifying the compound we employed glacial-acetic acid as a solvent. The purification consisted in collecting the nodular precipitate upon a filter and washing it with boiling alcohol. This will remove considerable benzoic-acid which contaminates the precipitate. Upon dissolving the benzoyl derivative and connecting with a steam-distilling apparatus the remanider of the benzoic-acid is removed, since it

is volatile with water-vapor. Part of the steam condenses as it passes through the solution and the water formed reprecipitates the benzoyl compound. The precipitate is again collected and washed with hot alcohol which eliminates the acetic-acid. Now by drying for six hours in a thermostat at 110° C we obtain the pure derivative.

The physical properties of these products are similar to the others formed in that they are amorphous and brown in color. The glucose being much lighter in color than the corresponding sucrose derivative. Liquefaction-points on the compound gave 125° to 128° for glucose and 188° to 192° for sucrose. Ignition on a platinum foil showed the presence of the benzoic-acid grouping by the dense clouds of soot formed. The glucose compound exhibiting more soot than the sucrose compound. Determinations for the elementary composition and subsequent hydrolysis for the estimation of benzoic-acid content were conducted until satisfactory checks were obtained. This will receive more attention in the succeeding pages.

From the general properties exhibited above we would presume that the glucose caramel derivative contained more benzoic acid in combination and consequently contained a greater portion of active hydroxyl groups than does the sucrose derivative. How far this view is confirmed by the analyses will be clearly seen from our data. Stolle reported that the compound which he obtained from benzoylating caramel was a mono- derivative. Contrary to this we find that the glucose contains at least six benzoyl groups while the sucrose has at least four. Since he used little pains in purifying his product and experienced such trouble in obtaining

the derivative it may explain the difference in the results secured. Though we performed the test with acetyl-chloride we did not purify or examine the compounds.

The next question of importance in the study of caramel is that of the cleavage products. Though at the present time little is actually known concerning these products, the mention of a few facts may afford a clearer view as to what takes place. Just as in the hydrolysis of sugars, where dilute hydrochloric and sulphuric acids react with about equal speed, so we find these two acids possessing the same activity towards caramel. Following Stolle's technique we digested portions of both glucose and sucrose caramels with 3% HCl upon a water-bath. Instead of removing after 18 hours we allowed them to digest for 30 hours. Both caramels gave a clear light-yellow solution, the sucrose slightly lighter, and yielded a brownish precipitate in the bottom of the digesting-flask. The glucose precipitate was much darker than was that from the sucrose caramel. If caramel existed as some polymeric carbohydrate then we would expect, upon hydrolysis with dilute acids, to obtain a hexose or at least some reducing sugar. This is exactly what happens. By treating the clear liquid, after filtration, with Fehling's solution a pronounced reduction is noted. As Stolle suggested, the sugar resulting from this hydrolysis can be isolated with methyl-alcohol but it requires considerable time. An osazone was formed upon treatment with phenylhydrazine and it appeared in the form of crystalline bundles or aggregates. The melting-point ranged between 188° and 190° C. Thus far our experimental results upon upon the clear solutions confirmed those secured by Stolle.

Upon concentrating the clear solution to small volume crystals appear. These possessed considerable sweetness and when added to Fehling's solution reduced it almost immediately. Both caramels acted similarily. Since we have a hexose in solution as a cleavage product we should expect to detect small amounts.of Laevulinic-acid. This is due to the action of the dilute acid which, during the long period of digestion, would tend to oxidize some of the sugar to Laevulinic-acid. Therefor we extracted a portion of the solution with ether, evaporated the ether solution and collected the thick liquid which remained. We crystallized a small amount and obtained the melting-point and then prepared the silver-salt of the compound. The examination of the silver-salt together with the physical properties proved conclusively that laevulinic-acid was present.

The appearance of laevulinic-acid at this stage is good evidence that there must be a hexa-carbohydrate.present, and that it may exist as the compound caramel or results from cleavage of the caramel.molecule. The residue remaining in the digestion-flask is humus-like. Nitric-acid oxidizes it to oxalic-acid. It is inscluble in all ordinary solvents. Ammonium hydroxide dissolves it to a considerable extent. These facts are characteristic of humus bodies. Apparently no difference exists in the cleavage products from the two caramels except in quantities formed.

Dilute nitric-acid oxidizes the caramels to saccharic, then to tartaric and racemic and finally to oxalic-acid. This process may be carried-out in the same manner as the preceeding experiment. After 24 hours digestion we obtain a clear solution

which is very light-yellow in color. No residue is left. The glucose caramel yields a much darker solution than does the sucrose. Each of these solutions were evaporated to dryness and then taken up with 100 cc of water; after neutralizing with potassium-carbonate and adding 25 cc of 50% acetic-acid they were again evaporated to small volume. Upon cooling the potassium salt of saccharic-acid separated from the solution. The procedure followed above for the isolation of saccharic-acid from the mother-liquor is that devised by Neuberg(1). Qualitative tests revealed traces of tartaric and large amounts of oxalic-acid. This oxidation by nitric-acid is common for all hexoses.

In the earlier pages we found that Sabaneeff mentions a peculiar fermentation which caramel undergoes. We, too, notice the same phenomena but a microscopical examination of the organism which grew in both of our purified caramels shows it to be a species of the Mucor molds. Apparently the caramel medium is satisfactory for its growth. The fact that it does not grow more readily in the undialyzed caramel solutions is due to the presence of para-formaldehyde in these solutions which inhibits the mold's growth.

A fair knowledge as to the characteristic reactions obtained with the two varieties of caramel has now been gained. The reactions suggest hexa-carbohydrates or polymeric forms which easily hydrolize to hexoses. A more rigid and system atic proof will be needed however before it can be definitely assured. In the succeeding division we will see that our results demand a new conception as to the molecular structure of caramel and the

(1)-Biochem' Zeitschrift. vol 28-355-1910.

relative size of the sucrose and glucose molecules.

(3).

MOLECULAR WEIGHT DETERMINATIONS AND EXAMINATION OF SUCROSE AND GLUCOSE CARAMEL BY MEANS OF SUBSTITUTED ORGANIC RADICLES.

Since previous investigators differed so pronouncedly as to the most likely empirical formula for caramel, we endeavored to secure some data in this regard by employing some of the more modern molecular-weight methods. Heretofore the empirical formulas seemed to depend essentially upon the elementary analyses. Sabaneeff(1) states that the molecular-weight is almost 3000. We are unable to see how he could have secured such astounding results. He neglects to state by what method these were obtained.

In our determinations we made use of the method devised by Eykmann(2) also the "Equilibrium Method"(3). This last mentioned method is, by far, the most satisfactory since caramel is so easily dissolved in water. Accurate checks were impossible, however, after making several analyses of the dialyzed caramels, we obtain an average from the results which varies but a few points from the actual values. The possibility of a large error is eliminated, owing to the number of determinations made. With the equilibrium method we secured the following values for the molecular-weights of the two varieties. Both the alcohol purified and dialyzed compounds were examined and the values included.

Glucose caramel--520, 541, 502, 530, 509, 496.
Average---516.
Sucrose caramel--659, 664, 630, 672, 654, 667.
Average---657.

(1)Jour. of Soc. Chem. Ind. vol 13 -1076 -1894.
(2)Chemiker Kalander by Biedermann. Zweiter Band. 1912.
(3)Zeitschrift fur Physik. Chem. vol 18 -572 -1895.

The elementary analysis of the glucose caramel gave-

Carbon,,.....47.09%	divide by	12.	= 3.92	-4.
Hydrogen..... 5.91	"	"	1.008= 5.91	-6.
Oxygen.....47.00	"	"	16.	= 2.94	-3.

Using the factors from the above percentage composition and the average molecular-weight for glucose caramel, 516, we calculate an empirical formula of $C_{20}\ H_{30}\ O_{15}$. This empirical formula demands a molecular-weight of 520.2 which is close to the average of our results. We note that hydrogen and oxygen are in the porportion to form water, thus establishing without doubt the carbohydrate nature of caramel.

Whether this is the actual formula is only a matter of conjecture. We might consider that glucose caramel possessed a polymeric hexose structure as $(C_6\ H_{12}O_6)_3$; this formula would call for a molecular-weight of 540. We are inclined to believe that the latter formula has a possibility for existence. The basis for our opinion is in the fact that the caramel is reduced to the form of a hexose by the hydrolysis by weak acids. However the caramel may exist as an anhydride and this in turn is hydrolyzed forming a hexose. We suggest the formula $C_{20}\ H_{30}\ O_{15}$. for glucose caramel as calculated from the forgoing experiments.

We did not determine the elementary analysis of sucrose since a satisfactory agreement in results is found from the earlier investigations. With this data-

Carbon.....	46.6%
Hydrogen.....	6.2
Oxygen.....	47.2

together with the average molecular-weight given above, 657, the following formula is derived - $C_{24}\ H_{42}\ O_{21}$. This formula is seen to possess a molecular-weight of 666, and agrees well with

that of Zsigmondy. From these conclusions it is evident that the question of the structure of caramel depends upon an accurate determination of the molecular weight. When this is known and has been corroborated then we can form logical deductions as to various reactions which take place.

HYDROLYSIS OF THE BENZOYL DERIVATIVE. The percentage of benzoic-acid present gives us knowledge of the number of benzoyl groups which have been substituted in place of the hydrogen of the hydroxyl. This percentage of the molecule occupied by the C_6H_5CO group is spoken of as the "benzoyl number" for that compound.

The procedure in hydrolysis was as follows. Accurately weighed portions, about 0.5 gm, of each caramel were heated with an excess of strong alkali (50%) upon a boiling water-bath for two hours. At the end of this period the excess of alkali was neutralized with pure phosphoric-acid and a slight excess of the acid added. By steam-distillation the benzoic-acid can be entirely removed from the caramel and caught in a flask under an upright condenser. The resulting benzoic acid is the titrated with standard $\frac{20}{N}$ barium-hydroxide using phenolpthalein as indicator. The point at which the above hydrolysis is complete is indicated by complete solution of the caramel. The benzoyl derivatives we had previously found to be very insoluble. The benzoic-acid cannot be titrated directly because of the caramel color.

In the case of glucose caramel benzoyl derivative we found for three determinations the following percentages and values-

	%Benzoic-acid	Benzoyl Number	Average
(1)	66.1	57.1	"B-N"
(2)	66.8	57.5	
(3)	65.3	56.9	57.16

For the sucrose caramel benzoyl derivative the following values were obtained-

	%Benzoic-acid	Benzoyl Number	Average
(1)	34.6	30.01	"B-N"
(2)	35.45	30.41	
(3)	35.9	30.8	30.40

The elementary analysis of these derivatives by the usual combustion method gave the tabulated results-

For the glucose caramel derivative:

Carbon.....64.38%
Hydrogen..... 5.17
Oxygen.....30.45

For the sucrose caramel derivative:

Carbon.....63.09%
Hydrogen..... 5.28
Oxygen.....31.63

A number of analyses were run on the above compounds and the percentages given above are the average. In either case the analyses checked quite close. Both of these derivatives burned at very high temperatures and it was found that a continuous current of oxygen yielded better results.

Making use of the above facts learned from the analysis we found that 57.16% of the resulting derivative of glucose caramel consisted of benzoic-acid and the previous molecular-weight determination for glucose caramel which gave 516, or rather using the empirical formula suggested above with a mole. weight of 520, a mathematical ratio is established between these compounds. Thus-

$$C_{20}H_{30}O_{15} + 6C_6H_5COCl = C_{20}H_{24}O_{15}(C_6H_5CO)_6 + 6\,HCl.$$

This formula for benzoyl derivative requires a molecular weight of 1158.4 and the percentage of carbon in the formula is 65%. This percentage is close to the value we found upon combustion of the derivative, viz 64.4%.

In similar treatment of the results from the sucrose derivative we find the following possible equation:

$$C_{24}H_{42}O_{21} + 4C_6H_5COCl = C_{24}H_{38}O_{21}(C_6H_5CO)_4 + 4HCl.$$

This formula gives a molecular-weight for the above derivative of 1103.4. Comparing the percentages of carbon in this empirical formula and the analytical results we find a respective ratio of 61.2% to 63.09%.

What these results may indicate must be interpreted with a great deal of deliberation. It shows that in the case of the sucrose caramel there are probably three hydroxyl groups acylated. Now whether or not any rearrangement of the molecule took place during the treatment with strong alkali and benzoyl-chloride is not definitely known. This strong alkali might affect a hydrolysis. This can be determined only after the molecular-weight of the benzoyl derivative is known. We have tried both the freezing-point and boiling-point methods but no reliable check-results were obtained. The benzoyl derivative is as difficult to work with as are the complex carbohydrates. We might say however that the figures gathered from the attempted molecular-weight determinations were much lower than our calculations above call for. This suggests that our opinion concerning an intra-molecular change within the caramel compound, when treated with strong alkali, may have a basis for justification. In considering this, we must bear

in mind the fact that after the hydrolysis of the benzoyl derivative was completed a solution was obtained bearing the color of the original caramel, which would hardly be expected if a rearrangement took place during the acylation. It is more possible that the grouping in the molecule is such that only four hydroxyl groups are capable of acylation under these conditions.

The glucose derivative presents a similar argument. Its high content of benzoic-acid was predicted from the comparison of the liquifaction-points and aromatic content of the two benzoyl compounds. Just why the glucose caramel should be more susceptible to acylation than the sucrose caramel will be left to the reader's deliberation until further light can be thrown upon this intensly interesting subject. We may state that our results are at variance with those of Stolle(loc.Cit), who reports that he obtained a tetra-acetyl but only a mono-benzoyl derivative from sucrose caramel. Part of this may be due to different methods of purification, since we feel that our method of dialysis gives absolutely pure caramel.

THE CARAMEL OSAZONE. The procedure followed in the preparation of these derivatives has been presented in an earlier part of this paper. We will only consider at this point the elementary analysis and the revelation it affords as to the probable carbonyl content of the caramel in question. Since we have been able to make nitrogen determinations upon only the glucose variety we will form no conclusions as to the sucrose caramel osazone except to mention that it possesses similar physical properties to the glucose.

The nitrogen determination was made by the usual absolute method, employing combustion-tube and nitrometer. Two analyses

of the purified osazone from glucose caramel gave the following percentages of nitrogen, 6.79 and 7.02%. Calculating from these percentages on the basis of the whole caramel molecule, we find that there are two phenylhydrazine groups taking part in this reaction, which suggests the following equation-

$$C_{20}H_{30}O_{15} + 3C_6H_5NH\,NH_2 = C_{20}H_{28}O_{14}(C_6H_5N\cdot NH_2)_2 + C_6H_5NH_2 + NH_3 + 2H_2O.$$

The above empirical formula demands a content of 6.96% of nitrogen. This value quite corresponds with the above determination. From this we would conclude that the caramel possesses, at the particular stage when phenylhydrazine reacts with it, at least one aldehyde or ketone group and that the usual oxidation occurs with the adjacent CHOH group, changing it to carbonyl. If this conception is true then the structure of the glucose caramel must either undergo extreme modification when treated with catalytic agents,or possess a closed chain of unsaturated carbon atoms. After a careful analysis of sucrose caramel osazone is made and the molecular weights of the two are determined, we will be in a position to state with more definitness as to the possible structure.

(4)

METHODS FOR THE DETECTION OF CARAMEL IN LIQUORS, ELIXERS, SYRUPS, EXTRACTS, VINEGARS, ETC.

A short discussion as to the reliability of the previous tests employed by food-chemists is of value at this point.

The much used method of Amthor(1) where paraldehyde and absolute alcohol are employed to reveal traces of caramel in various food-stuffs, has been proven to our satisfaction to be absolutely unreliable. Especially is this emphasized in cases of very dilute solutions of caramel. The efficiency of this test depends upon the statement that paraldehyde destroys the color of caramel and causes a precipitate to settle on the sides of the test-glass. This precipitation of caramel by paraldehyde has no significance chemically and is not distinctive for caramel since other compounds of similar structure cause the same phenomena. In the second place, after repeated trials, we found that glucose caramel, upon dilutuon to the depth of color usually found in commercial adulterations, gave such a faint precipitate that it might easily be overlooked in routine work. Then again, when applied to sucrose caramel the efficiency of the test is reduced. Sucrose caramel diluted to a similar coloration as in the above experiment with glucose, failed to respond to the test. It was only upon more concentrated solutions that the reaction occurred.

The test put forth by Woodman and Newall, where an alkaline solution of $ZnCl_2$ is used to precipitate the caramel and other coloring-matters together with occluded substances, meets a similar fate. In making this statement we base our opinion on

(1) Jour. Soc Chem Ind. vol 4 -361 -1887.

the inefficiency of the subsequent confirmatory tests after the coloring matter is precipitated from the substance to be analyzed. These confirmatory tests were paraldehyde and formation of an osazone, or rather a precipitate with phenylhydrazine. We have mentioned the inadequacy of the paraldehyde reaction. Respecting the phenylhydrazine reaction we will simply state that if caramel alone was present then the formation of the caramel osazone would be a characteristic and distinctive test; but we know that should other organic coloring-matters or vanillin or perhaps reducing-sugars, be present, then a similar reaction would be experienced. It is evident that this method cannot be deemed satisfactory.

We have tried tests for the efficiency of Fuller's earth and find it of no consequence. This was in response to the reported method whereby Fuller's earth was expected to absorb the artificial vegetable coloring-matter, caramel.

It is clearly evident from what has been written that the chemist meets one of the most difficult fields when he attempts to analyse for coloring-matter. As yet the field is but entered. A practical method for the determination of caramel would aid in the removal of one opposing obstacle.

We have sought to devise a workable method from our present limited knowledge of caramel but a simple method seems impossible. By a combination of characteristic reactions and properties one is able to evolve a reliable qualitative test for its presence. Before concluding this paper we will outline a method which we believe will prove satisfactory for most cases caramel adulteration. At present we wish to discuss one more method -that

advanced by G.H.Lichthardt(1). His test is the most characteristic for very dilute solutions of pure caramel. What the reaction is we cannot definitely state for it appears to be unknown. The reaction consists in adding a slightly acid solution of tannic-acid to a solution of caramel, wherupon a very flocculent precipitate appears and the entire coloring power of caramel is destroyed. This test is not of value for extracts where vanillin is present, for a similar precipitate is noted. In whiskeys and brandeys it is just as unreliable. A precipitate is formed by compounds in the pure whiskey or brandy therefor a more convincing test must be used.

In considering whiskeys and brandy, wines, vinegar, etc where long storing in"fired" or "charred" white-oaken casks take place a very interesting problem presents itself. In charring,a smudge is made from the refuse shavings and accumulating wood in the manufacture of the casks. The the casks are usually inverted over the burning shavings and the distillation products from the wood permeate the pores of the casks and remain, only to be dissolved by the stored contents. At the same time of the charring a sufficient heat is produced to cause some of the carbohydrate material found in the white-oak wood to char. These charred substances together with the cresols, tannin,resins, etc constitute the coloring matter of the whiskeys and brandy. The alcohol and water present form excellent solvents and when allowed to work from two to ten years cause a solution of most of the distillation products. Since the wood-fibers contain carbohydrat

(1) Journal of Ind and Eng Chem. vol2 -389 -1910.

material, we are beginning to believe that a part of these soluble coloring matters resulting from the charring are nothing more nor less than caramels, characteristic of their sources.

Hence, in choosing a test for added caramel in the presence of this multiplicity of compounds, considerable judgement must be used. It is not an impossibility but nevertheless it proves that the chemist cannot expect to accomplish such an analysis in a few minutes of time. We have performed some interesting tests with white-oak wood used in the manufacture of casks for distillerys, etc, and from the results of these experiments the above conclusions were evolved. After charring some of the oak wood with shavings secured from the same source, it was placed first in a solution of water and later in a mixture of alcohol and water(30%). The extraction of the char was allowed to continue for two weeks. At the end of this period a dark-yellow solution resulted, suggesting the color of amber. This solution was tested with all of the methods in vogue for the detection of caramel and all responded; some to a high degree and others only perceptibly. With basic lead-acetate, benzoyl-chloride, tannic-acid test solution, paraldehyde and stannous-chloride, a decided precipitate resulted. Thus the seeming futility of presenting any satisfactory method for the detection of added amounts of caramel. By making use of the method described in the following paragraph we were able to find only a trace of caramel, a very slight trace; however there was evidence that a small amount of caramel must exist preformed in properly cured whiskey. Large amounts would show adulteration.

We now submit the following method to chemists for the identification of caramel in substances where coexisting compounds hamper its detection:

Make the substance to be analyzed strongly alkaline, add benzoyl-chloride in small volume -1 cc to 10 cc of the substance. Shake thoroughly until the odor of benzoyl-chloride ceases to be detected. A heavy precipitate will result. If only sugars are present a soft spongy mass, light in color appears. If higher compounds such as tannin, vanillan, caramel etc,are present a heavy brownish nodular precipitate is secured. Now the benzoyl derivatives of sugar are extremely soluble compounds when compared with those of caramel. According to Vaubel(1) the benzoyl derivative of tannin is easily soluble in acetone or chloroform; the benzoyl derivative of vanillin is also very soluble while in the early investigations we found that glacial-acetic acid was the only satisfactory solvent for the caramel derivative, it being insoluble in everything else excepting benzol and nitro-benzol and these only slightly. This insolubility was attributed to its amorphous qualities. With these facts in hand the outlining of this method is extremely simple. When we secure our benzoyl precipitate we need only to expect caramel if it shows a trace of brown color. The precipitate is now extracted with hot alcohol to dissolve the derivatives due to sugar, vanillin and resins should they be present. Filter and if a residue is left we may expect either caramel or tannin or perhaps both. Now extract the dried precipitate with chloroform or acetone for some time. If no

(1) Vaubel II . page 159.

color is dissolved by the chloroform or acetone and a brown residue still remains, then caramel was present and the residue consists of the benzoyl derivative of that compound. If the entire precipitate is dissolved then no caramel is present. Should only part of the residue be dissolved by the acetone or chloroform, filter, add a small volume of acetone and extract for more tannin. If no more is extracted and a brown residue is still noted, then both caramel and tannin were present. The efficiency of the test depends upon careful extraction with the solvents employed. When followed out in detail it seems thoroughly reliable.

Should one care to examine the caramel residue further for confirmation, a small quantity of alkali may be added to the residue, whence the benzoyl derivative will be hydrolyzed and the solution become brown owing to the soluble caramel formed. This solution may now be neutralized with phosphoric-acid and a solution of phenylhydrazine-acetate added in small amounts. An immediate precipitate of non-crystalline structure confirms the presence of caramel. Ordinarily this latter confirmatory test will not be necessary after a few trials with the solubility test. In employing this method a moderately large portion of the substance must be used; the amount will vary according to the coloration of the substance in question. We have applied the above test to a mixture of tannin, vanilla extract and caramel and it yielded splendid results.

SUMMARY.

(1). We have ascertained that caramel is a definite compound appering as an intermediary product in the dry distillation of sugars, along with a long series of other compounds.

(2). A difference of structure and molecular constitution was found to exist between the caramels prepared from glucose and sucrose.

(3). From the elementary analyses and molecular-weight determinations we suggest the following empirical formulas for the purified caramels-

Sucrose caramel..... $C_{20}H_{30}O_{15}$.
Glucose " ..... $C_{24}H_{42}O_{21}$.

(4). The substitution of the organic radicle, C_6H_5CO, for the hydrogen of the hydroxyl shows sucrose caramel to contain four active hydroxyl groups and glucose to contain six.

(5). We are satisfied that the caramels form typical osazones. The analysis of the glucose caramel osazone shows that two phenylhydrazine radicles react with the caramel and proves that at least one carbonyl group is present. The actual significance of these analyses awaits accurate molecular-weight determinations of the examined compounds.

(6). The tests submitted for the identification of caramel in compounds and mixtures of substances have been found very unsatisfactory and unreliable.

(7). We have outlined a method for detecting caramel which depends upon the insolubility of the benzoyl derivative and the subsequent confirmation when isolated from contaminating substances.

A wider view of the structure of caramel can be gained only by careful outlined methods of attack. It is not an impossibility as previously conceived, but will be found to be an exceedingly pleasing abd interesting subject for research. We believe that a thorough knowledge of the compound caramel and an appreciation of the simultaneously formed compounds will greatly enlarge our conception of the sugar molecule.

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